KINETIC RESOLUTION OF SECONDARY ALCOHOLS WITH COMMERCIAL LIPASES: Application to Rootworm Sex Pheromone Synthesis

PHILIP E. SONNET and MARY WELCH BAILLARGEON

Animal Biomaterials Research Unit
Agricultural Research Service, USDA
Wyndmoor, Pennsylvania 19118

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Abstract—The relative rates of enzyme-catalyzed esterification of the enantiomers of 2-octanol with various acids were determined for several commercial lipases. Interesterifications and hydrolyses of racemic 2-octanol esters catalyzed by these enzymes were also examined. Novo's *Mucor miehei* lipase exhibited considerable enantioselectivity and was therefore employed to prepare 8-methyl-2-decanols with high configurational purity at the carbinol carbon. Esters of these alcohols had been previously identified as sexually attractive to several rootworm (*Diabrotica*) species, and the stereochemistry of those esters had been shown to be critical to the attraction. The enzymatic resolution provides a convenient method to obtain such esters in a desired state of configurational purity.

Key Words—*Diabrotica* sp., kinetic resolution, lipase, methyl carbinol, *Mucor miehei*, pheromone, western corn rootworm, Coleoptera, Chrysomelidae.

INTRODUCTION

The relationship of insect pheromone stereostructure to insect behavior has received considerable attention (Brand et al., 1979). Successful completion of a pheromone identification and evaluation of methods by which to control the behavior of an insect pest often are dependent on the ability to prepare pure stereoisomers. Therefore there is a continuing interest in the development of novel, or improved, syntheses of stereocenters that can be incorporated into structures that are commonly found in pheromone blends. Additionally, such new methodology often serves the broader interest of organic synthesis generally. Among the approaches commonly used for asymmetric synthesis, enzy-

matic resolution can perform quite well providing (1) a preparation is commercially available or can be reproduced easily in a laboratory, and (2) the precursor of the target stereocenter mimics the enzyme's normal substrate adequately to provide reasonable reaction rates. Enzymatic conversions that have been targeted for chiral insect sex pheromone structures include *N*-deacylation of racemic acetamido acids with an *Aspergillus acylase* (Mori and Iwasawa, 1980; Sugai and Mori, 1984), selective hydrolysis of alkynol esters by cultures of *Bacillus subtilis* var. *Niger* (Mori and Akao, 1980), reduction of β-ketoesters and ketones with Baker's yeast (*Saccharomyces cerevisiae*) (Mori, 1981; Mori et al., 1985), and the reduction of α-methyl-α,β-unsaturated aldehydes to chiral α-methylcarbinols also with Baker's yeast (Gramatica et al., 1985). In all of these cases an enzyme preparation of previously documented properties was examined with a selected group of substrates. We report here a study of a number of commercial lipases in which stereoselective hydrolysis, esterification, and transesterification of 2-octanol as a typical 2-*n*-alkanol were evaluated. The information was then used to synthesize 8-methyl-2-decanol samples in which the carbinol carbon was $\geq 97\%$ (94% *ee*) configurationally pure. Esters of such alcohols are pheromones of various *Diabrotica* species (Guss et al., 1985).

METHODS AND MATERIALS

Gas–liquid chromatography (GLC) was performed with a Shimadzu GC-Mini-2 instrument using an SPB-1 column (0.25 mm ID $\times$ 30 m) operated at temperatures indicated below with a 50 : 1 split ratio and He carrier gas. Infrared (IR) spectra were obtained on a Perkin-Elmer 1310 Spectrophotometer (3% solutions in CCl_4 or $CHCl_3$). Mass spectra (MS) were recorded with a Hewlett-Packard HP-5995 GC-MS system employing an OV-1 capillary column (0.25 mm ID $\times$ 30 m). Nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ using a JEOL JNM-GX 400 FT NMR spectrometer. Free fatty acid titrations were performed with a Radiometer pH instrument comprised of an ABU-80 Autoburette module operated in "end point" or "pH stat" modes as appropriate. Lipases were obtained from commercial sources and were employed directly without any purification. Specific lipases and manufacturers are described in text and tables; additional information follows: Gist-Brocades USA, Inc., Charlotte, North Carolina 28224; Amano International Enzyme Co., Inc., P.O. Box 1000, Troy, Virginia 22974; Novo Laboratories, Inc., P.O. Box D, Wilton, Connecticut 06897-0820. The α-methylbenzyl isocyanates, both *R* and *S*, were synthesized from the corresponding amines that had been purchased from Hexcel Corp., using the method of Pirkle and Hauske (1977). The α-methylbenzylamines were 98.7% *R* and 99.4% *S*, but the analyses reported here were not corrected. The actual ratios were in fact slightly higher than those reported in this paper.

Rate ratios are calculated from the expression:

$$\frac{k_A}{k_B} = \frac{\ln (1 - C)(1 - ee)}{\ln (1 - C)(1 + ee)}$$

wherein C is fraction conversion and ee is enantiomeric excess of starting material expressed as a fraction (Martin et al., 1981). For hydrolysis the stereobias was evaluated in the product and,

$$ee_a = \frac{C(ee_b)}{1 - C}$$

wherein a and b represent starting material and product, respectively.

The 2-octanol was purchased from Aldrich Chemical Co.; other reagents and solvents were of commercial origin and employed directly except for esters of 2-octanol and 8-methyl-2-decanol. The esters were synthesized in the usual manner from the alcohols using acid chlorides and pyridine solvent. They were distilled and characterized by IR, MS, and GLC.

Initial Rate Assay. Weighed amounts of commercial lipase were allowed to react in an emulsion created by brief sonication of 10% (w/w) purified olive oil and 10% (w/w) gum arabic. Five milliliters of emulsion, 0.2 ml of 2.85 M NaCl, and 30–100 μl of aqueous enzyme were brought together at ambient temp. Free fatty acid release was measured by titration with 0.100 N NaOH using "pH stat" mode at pH 7.3 or other indicated pH (Table 1). Enzyme

TABLE 1. INITIAL RATE ASSAYS OF COMMERCIAL LIPASES ON OLIVE OIL[a]

Lipase	Manufacturer code	Rate (μmol/min/mg)	Position selectivity[b]	Fatty acid selectivity[c]
Aspergillus niger, 1	Amano-AP	0.154	1,3	18 (*cis*-Δ9)
Aspergillus niger, 2	Amano-K	11.2	1,2,3	10,12
Candida rugosa	Enzeco	9.82	1,2,3	18 (*cis*-Δ9)
	Sigma	9.70	1,2,3	18 (*cis*-Δ9)
Mucor miehei	Amano-MAP	2.67	1,3	<12
	Gist Brocades-S	40.0	1,3	<12
	Novo (powder)[d]	5.33	1,3	<12
	Nova 3A (resin)	0.095	1,3	<12
Porcine pancreatic[e]	Sigma	15.6	1,3	4
Rhizopus arrhizus[f]	Gist Brocades	33.4	1,3	8,10

[a] pH maintained at 7.3 unless otherwise indicated.
[b] Position selectivity with respect to triglyceride hydrolysis.
[c] Fatty acid selectivity (Brockerhoff and Jensen, 1974; Borgstrom and Brockman, 1984); the preferences are slight in most cases.
[d] The powder form is not currently available, although the lipase is now formulated on both an ion exchange resin sold as "3A" and as a solution as "225".
[e] pH 8.0 with 0.2 ml of 0.3 M $CaCl_2$ to 5 ml emulsion (Methods and Materials).
[f] 0.2 ml of 0.3 M $CaCl_2$ to 5 ml emulsion.

solutions were prepared in distilled water with additives as indicated. The conditions were chosen to optimize activity of the lipase on the olive oil. Initial rate assays provide reproducible data by which comparisons of enzymatic activity on a given substrate may be made.

α-Methylbenzyl Carbamates. The alcohols, and reaction mixtures that were being analyzed for enantiomeric alcohol content, were derivatized by reaction with excess (*R*)- or (*S*)-α-methylbenzyl isocyanate (for example, 10 μl of neat racemic alcohol plus 20 μl of isocyanate) at 50–60°C for 0.5 hr in a screw-cap vial. The excess isocyanate was discharged by adding a few drops of methanol and warming at 50–60°C for 0.25 hr. The resulting mixture was diluted with acetone and filtered through Na_2SO_4 in a disposable pipet, if necessary, for GLC analysis. Spectral and chromatographic data for the carbamates follow: (1) 2-octanol *N*-α-methylbenzyl carbamate: IR (CCl_4) 3460, 1715 cm^{-1}; EI-MS (*m/e*) 277 $(M)^+$, 262 $(M\text{-}CH_3)^+$, 164,150; GLC (220°C) k′ 3.58 (*R*S**), 3.77 (*R*R**), $\alpha = 1.054$; (2) 8-methyl-2-decanol *N*-α-methylbenzyl carbamate: IR (CCl_4) 3460, 1715 cm^{-1}; EI-MS (*m/e*) 319 $(M)^+$, 164,150; [^{13}C]NMR ($CDCl_3$): 11.4, 19.2, 20.3, 22.6, 22.7, 27.0, 29.5, 29.9, 34.4, 36.3, 36.5, 50.5, 71.6, 116.6, 125.9, 127.2, 128.6, 155.7 ppm. GLC (240°C) k′ 3.73 (*R*S**), 4.00 (*R*R**), $\alpha = 1.072$.

Lipase Screening for Kinetic Resolution. Racemic 2-octanol (0.33 g, 2.5 mmol) was dissolved in 10 ml of nanograde hexane. The organic acid (2.5 mmol) was added as was 1.0 g of the lipase powder (2.0 g of porcine pancreatic lipase powder). The mixture (heterogeneous) was stirred for a period of time in an incubator at a specified temperature (Tables 2–5), diluted with ethanol, and immediately titrated to pH 9.5 with 0.1 N NaOH. Blanks were obtained for each lipase employed. The titrated mixture was partitioned with hexane, and

TABLE 2. ESTERIFICATION OF (±)-2-OCTANOL WITH *Candida rugosa* LIPASE[a]

Acid	Time (hr)	C[b]	ee[c]	k_R/k_S
Acetic	20	0	—	—
Propanoic[d]	20	76.0	<3	ca. 1
Octanoic	20	84.8	12.8	1.14
Dodecanoic[d]	3.5	17.2	<4	<1.5
	6.5	43.2	6.5	1.14
	20	85.8	16.6	1.19
	(20°C) 22	61.7	20.4	1.53
	(4°C) 22	29.9	13.0	2.12

[a] Enzeco's lipase suspended in hexane at 40°C unless otherwise indicated.
[b] Percent conversion.
[c] Enantiomeric excess of residual 2-octanol.
[d] Results with Sigma's lipase virtually identical.

TABLE 3. ESTERIFICATION OF (±)-2-OCTANOL WITH PORCINE PANCREATIC LIPASE[a]

Acid	Time (hr)	C^b	ee^c	k_R/k_S
Propanoic	70	17.2	6	1.9
Octanoic	48	51.8	56	5.4
2-Ethylhexanoic	70	0.9	—	—
Dodecanoic	48	52.7	66	7.6
Tetradecanoic	70	53.8	66	7.0
Hexadecanoic	70	50.4	52	5.1
Octadecanoic	70	46.2	54	7.4
9-Octadecenoic[d]	70	55.0	78	10.4
Methoxyacetic	70	0	—	—
2-Chloropropanoic	70	0	—	—

[a] Sigma's lipase suspended in hexane at 40°C.
[b] Percent conversion.
[c] Enantiomeric excess of residual 2-octanol.
[d] Cis-isomer.

TABLE 4. ESTERIFICATION OF (±)-2-OCTANOL WITH LIPASES OF *Rhizopus arrhizus* AND *Mucor miehei*[a]

Acid	Time (hr)	C^b	ee^c	k_R/k_S
R. arrhizus				
Propanoic	70	63.8	ca. 13	ca. 1.3
Octanoic	70	14.6	12	6.4
Dodecanoic	70	8.5	10	>10[d]
9-Octadecenoic (*cis*)	70	19.6	16	5.6
M. miehei				
Propanoic	70	15.8	9.5	3.4
Octanoic	70	39.1	66	>100
Dodecanoic	70	43.5	66	25.7
Propanoic[e]	70	18.2	14	5.0
Octanoic[e]	70	49.7	74	15.3
Dodecanoic[e]	70	46.1	67	16.4

[a] *R. arrhizus* lipase from Gist Brocades; *M. miehei* lipase powder from Novo; 40°C in hexane.
[b] Percent conversion.
[c] Enantiomeric excess of residual 2-octanol.
[d] Value of C too low to make ratio calculation accurate.
[e] Small amount of water added initially, namely 1 μl of pH 6.0 phosphate buffer, which is equivalent to ca. 2% of that which would be generated by complete conversion.

TABLE 5. ESTERIFICATION OF (±)-2-OCTANOL WITH OCTANOIC ACID USING OTHER LIPASES[a]

Lipase	Time (hr)	C^b	ee^c	k_R/k_S
A. niger, 1[d]	120	4.8	—	—
A. niger, 2[e]	120	59.6	83.6	9.1
M. miehei[f]	46	7.5	—	—
M. miehei[g]	70	42.4	26.8	2.8
M. miehei[h]	90	40.8	72.6	>100

[a] Reactions were conducted in hexane at 40°C. Two additional lipases produced very low conversions (*R. niveus*: Amano-N, *R. oryzae*: Amano-FAP).
[b] Percent conversion.
[c] Enantiomeric excess of residual alcohol.
[d] Amano-AP.
[e] Amano-K.
[f] Amano-MAP.
[g] Gist Brocades-S.
[h] Novo-3A.

the hexane layer was washed 2× with equal volumes of water. The organic phase was dried ($MgSO_4$) and concentrated; the resulting product mixture was then derivatized for GLC analysis of residual alcohol. The esters present were also observed sometimes but did not interfere. Hydrolyses and transesterifications were conducted and analyzed in a similar manner and are reported in Tables 6 and 7. Larger amounts of enzyme were required to effect hydrolysis. The following procedures are representative.

Kinetic Resolution by Hydrolysis. (*R*)-2-octanol from racemic 2-octyl octanoate, the ester (5.12 g, 20.0 mmol) was vigorously stirred in 20 ml of distilled water at room temperature containing 1.0 g of Novo's *Mucor miehei* lipase powder. The pH was maintained at 7.0 with 2 N NaOH. After 2 days, 1.10 ml of base had been consumed (11% conversion). Ethanol (50 ml) was added, and the mixture was extracted with 3 × 50 ml of hexane. The extract was washed with 2 × 30 ml of water, dried ($MgSO_4$), and concentrated. Distillation of the residue produced 0.27 g of 2-octanol that was 98.0% *ee* (*R*): bp 90°C/30 mm. Further hydrolysis (another 12.5% conversion) gave an additional 0.1 g that was 97% (*R*).

For (*R*,*S*)-8-Methyl-(*R*)-2-decanol from racemic octanoate ester, the ester (5.95 g, 20 mmol) was stirred in 20 ml of Novo-225 after brief sonication. Novo-225 is a solution of glycerol-stabilized *M. miehei* lipase. The mixture was maintained at room temperature and at pH 7.0 until 25–30% conversion had occurred (24 hr). The product was worked up as above to give 0.67 g of 8-methyl-2-decanol that was 97.6% *ee* at the carbinol carbon: bp 54–70°C/0.2 mm.

TABLE 6. HYDROLYSIS OF ESTERS OF (±)-2-OCTANOL[a]

	C[b]	ee[c]	Time (hr)	k_R/k_S
C. rugosa (Enzeco)				
Propanoate	30	4		
Octanoate	30	8		
Dodecanoate	21	0		
M. miehei (Novo powder)				
Propanoate	22.7	92	72	31[d]
Octanoate	18.7	97.8	20	>100
Dodecanoate	8.0	95	22	47
(±)-8-Methyl-2-decanol				
Propanoate	8.8	96.8	18	60[e]
Octanoate	15	98.7	90	>100[e]

[a] Reactions were conducted at ambient temp at pH 7.0, no additives.
[b] Percent conversion.
[c] Enantiomeric excess of product alcohol.
[d] The rate was 59 for this reaction using the liquid form of the lipase, Novo-225.
[e] Novo-225.

Kinetic Resolution by Esterification. For 8-methyl-(*R* and *S*)-2-decanols, the racemic alcohol (4.51 g, 26.2 mmol) was dissolved in 45 ml of hexane containing 9.43 g (65.5 mmol) of octanoic acid. Novo's lipase 3A (5.0 g) was added, and the heterogeneous mixture was sitrred at 30°C for six weeks. The progress of the reaction was followed by GLC monitoring of the residual alcohol enantiomer content, and the reaction was worked up by filtration to recover enzyme. The filtered solution was washed with aqueous base and then water. After the organic phase had been dried, the solvent was removed and the alcohol distilled [bp 65–72°C/0.1 mm, 1.83 g of 94.6% 2(*R*)]. Distillation was continued until the head temperature was 120°C. This produced ca. 0.2 g that was discarded. The pot residue was saponified with 33 ml of 6 N KOH and 50 ml of MeOH under reflux overnight. The saponified material was extracted into hexane and processed as above. Distillation produced 1.88 g of 93.2% 2-(*S*)-alcohol.

Recovered lipase was exposed competitively with fresh Novo-3A in an esterification of 2-octanol with octanoic acid. The recovered enzyme preparation had retained ca. 85% of its activity.

RESULTS AND DISCUSSION

Lipases are a class of enzymes that hydrolyze natural triglyceride mixtures (animal fats and vegetable oils) to diglycerides, monoglycerides, glycerine, and fatty acids. Excellent reviews have been provided of their purification, char-

TABLE 7. INTERESTERIFICATIONS

Lipase and ester	Time (hr)	Temperature (°C)	C^a	ee^b	k_R/k_S
C. rugosa					
Tributyrin	24	25	30–40	6	ca. 1
Tributyrin[c]	24	25	ca. 5	4	ca. 1
Tributyrin[c]	48	25	10–15	4	ca. 1
Methyl methoxyacetate	24	40	0	—	—
Methyl α-chloropropanoate	24	40	>50	<4	1
Olive oil	20	40	100	0	—
Olive oil	4	40	58	0	1.0
Porcine pancreas					
Tributyrin	70	40	40	38	5
Olive oil	20	40	54	39	2.8
M. miehei (Novo powder)					
Tributyrin	70	40	50	72	13
Tributyrin[d]	20	30	29	26	5.8
Trioctanoin[d]	24	30	28	6	1.4
Olive oil[d]	24	30	47	28	2.5
Tallow[d]	24	30	47	26	2.5
Tributyrin[e]	20	30	32	50	>100
Tributyrin[f]	20	30	16	24	>100
Tripropionin[f]	168	40	50	83	28
Tallow[f]	72	40	60	71	6

[a] Percent conversion.
[b] Enantiomeric excess of residual alcohol.
[c] Chromosorb 101 (Cambou and Klibanov, 1984a).
[d] Hexane solvent.
[e] One μl of water.
[f] Hexane solvent plus 1 μl of water.

acterization, and catalytic action (Brockerhoff and Jensen, 1974; Borgstrom and Brockman, 1984). In addition to pork pancreatic lipase that is available as a by-product of the swine industry, a number of fungal lipases have been made available to benefit the dairy and cheese industries. Considerable interest exists in enzymatic restructuring of natural triglycerides and in enzymatic cleavages on an industrial scale. The outlook for lipase availability as catalysts for general organic synthesis therefore seems positive, although their potential has not yet been fully explored.

Triglyceride lipases are uniformly lacking in stereoselectivity in hydrolysis of triglycerides (Brockerhoff and Jensen, 1974, pp. 56–58). Nevertheless, stereobiases of varying degree are exhibited by these enzymes in esterification, transesterification, and hydrolysis of other substrates. Lipases of *Candida rugosa* (Cambou and Klibanov, 1984a,b; Langrand et al., 1985; Laumen and

Schneider, 1984; Satoru et al., 1985), porcine pancreas (Lavayre et al., 1982; Ladner and Whitesides, 1984; Wang et al., 1984), *Pseudomonas aeruginosa* (Hamaguchi et al., 1984, 1985), and *Rhizopus* sp. (Laumen and Schneider, 1984) have been variously employed to effect kinetic resolutions or to selectively esterify diols that contain stereoisotopic alcohol groups. In addition there is an indication that lipases may exhibit a stereobias in transesterification, therefore likely esterification as well, with alcohols that closely resemble diglycerides (Sonnet et al., 1986).

With this information in mind we wished to screen available lipases for possible utility in preparing 8-methyl-2-(*R* and *S*)-decanol, the parent alcohol of a pheromone structure that is common to a number of diabroticites (Guss et al., 1985). Several of these species are serious pests of corn, and the ability to monitor selectively may depend critically on the stereochemistry of the bait. Current knowledge indicates that the configuration of the methyl-bearing hydrocarbon center must be *R* for all species; the *S* configuration is inactive. Hence this center may be racemic in chemicals intended to attract with no disadvantage beyond dilution. The carbinol center, however, is species differentiating. Moreover, evidence has accrued that indicates that several percent of antipodal configuration of a pheromone can inhibit response (Brand et al., 1979). Since racemic 8-methyl-2-decanol can be readily prepared in several ways (Sonnet et al., 1985), including a procedure developed for the USDA by Zoecon Corporation,[1] an efficient enzymatic resolution of the racemic carbinol center might be a useful accessory reaction.

Initially 2-octanol and its esters were exposed to various reaction conditions using commercial lipases. The enzymes were calibrated for activity on olive oil (ca. 85% triolein) using an "initial rate assay" (Methods and Materials). The results are given in Table 1. Considerable differences exist in the lipase activity of these materials. In part this is attributable to the manner and degree of purification of each commercial preparation as well as to potential intrinsic differences. The lipase of *Candida rugosa* (previously named *C. cylindracea*) offered by Sigma is, we learned later, supplied by Enzeco, and the initial rate measurement of activity and subsequent evaluation of stereobias in resolutions were consistent with this. However, great differences in activities toward olive oil were exhibited by the several commercially available lipases of *Mucor miehei*. Moreover, the degrees of stereobias exhibited (see below) were also different. Because of the heterogeneous character of these preparations, only a more probing study of the protein content and knowledge of the (proprietary) preparation of the lipase could provide a definitive answer to questions concerning the enzymes themselves. We have opted therefore to treat these materials simply as undefined but, if reproducibly prepared, potentially useful catalysts for organic synthesis.

[1] Specific Cooperative Research Agreement No. 58-7B30-1-176, August 14, 1981.

Esterification. The alcohol, 2-octanol, was allowed to react in hexane with an organic acid at a selected temperature for a period of time in the presence of the lipases. Residual free fatty acid was determined by titration and the enantiomeric composition of the residual alcohol was determined by conversion to carbamates with (*R*)- or (*S*)-α-methylbenzyl isocyanate (Methods and Materials) (Pirkle and Hauske, 1977). Although a number of chiral derivatizing agents for secondary alcohols have been employed (Doolittle and Heath, 1985), the carbamates could be formed directly from crude reaction products, and separations of the diastereomers of 2-octanol and 8-methyl-2-decanol were excellent (Figures 1 and 2). The rate ratio, k_R/k_S could then be calculated (Methods and Materials). The results employing *C. rugosa* lipase (Table 2) were disappointing; the rate ratios were very low although they could be improved by lowering the reaction temperature. Porcine pancreatic lipase (Table 3) proved to be more selective and demonstrated the validity of varying the fatty acid as a means to optimize the selectivity. The lipase of *Mucor miehei* (Novo powder) showed only low selectivity with propanoic acid, but very high selectivity with octanoic acid (Table 4). *Rhizopus arrhizus* lipase was rather unreactive with acids of medium and long chain length as well as only modestly selective (Table 4). Other lipases were evaluated with octanoic acid only, and the results are given in Table 5.

Summarizing these results, *C. rugosa* lipase catalyzed relatively fast esterifications of 2-octanol but with low selectivity. Porcine pancreatic lipase acted more slowly (lauric acid fastest) with greater discrimination. *R. arrhizus* lipase acted slowly also (propanoic acid fastest). *M. miehei* lipase (Novo only) provided greatest discrimination between the enantiomeric 2-octanols. Temperature affects both the overall rate and the relative rate; and α-branching of the acid (see Table 2) dramatically lowers reactivity. Initial presence or absence of small amounts of water affects results as well (Table 4). Since esterification produces water, the observed results starting "dry" must be a composite. Fastest reactions may occur with shorter or longer acid chains, but stereobias (invariably $R > S$) was always poorest with shorter acid chains.

Hydrolysis. The propanoate, octanoate, and dodecanoate esters of 2-octanol were arbitrarily chosen for exposure to lipases. The pH was automatically maintained at 7.0 and the titration thereby indicated instantaneously the degree of conversion. As with the esterifications, the crude reaction mixture was derivatized and analyzed for enantiomeric excess of the alcohol, which was this time the product. Rate ratios were calculated again and the results for *C. rugosa*, *R. arrhizus*, and *M. miehei* lipases are given in Table 6. All of the lipases reacted very slowly and, although emulsifying agents were not specifically added, Novo-225, (*M. miehei* lipase intended for direct lipolysis of triglycerides) reacted no more readily with 2-octanol octanoate than did the Novo powder. This implies that the enzymes are inherently slow in their reactions with esters 2-octanol. Nevertheless, the Novo lipase (both powder and Novo-225)

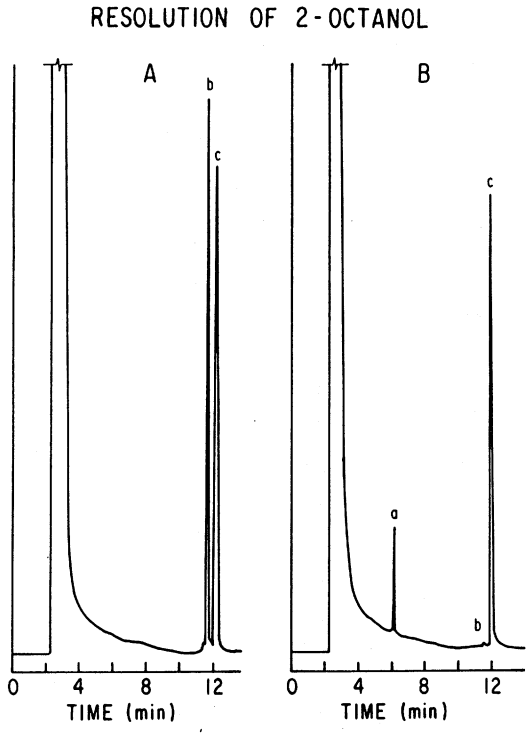

FIG. 1. (A) Analysis of α-methylbenzyl carbamates found by the reaction of (±)-2-octanol with (*R*)-α-methylbenzyl isocyanate by GLC using an SPB-1 capillary column (220°C). Peak b is the *RS* diastereomer; peak c is the *RR* diastereomer. (B) Analysis of 2-octanol obtained by hydrolysis of the racemic octanoate ester using *M. miehei* lipase (Novo, powder). Peak a is contamination by the ester, peaks b and c are the diastereomeric derivatives as in A. The major enantiomeric alcohol formed has the *R* configuration.

did show strong stereoselection, particularly for the octanoate ester; the *R*-esters reacted preferentially whenever bias was noted.

Transesterification. This process appears to be receiving considerable attention in the fats and oils industries. There the application is intended to produce new triglyceride mixtures by displacing existing acid residues with new ones to generate materials with more desirable physical properties. Stereochemistry probably plays an insignificant role in determining gross physical properties compared to positional isomerism on the glycerol moiety. However, one might use a fat or oil as a very cheap source of acid residues for kinetic resolutions in which acid residues are being transferred from glycerol to another (racemic) alcohol.

Reactions of 2-octanol with a selection of esters including triglycerides as

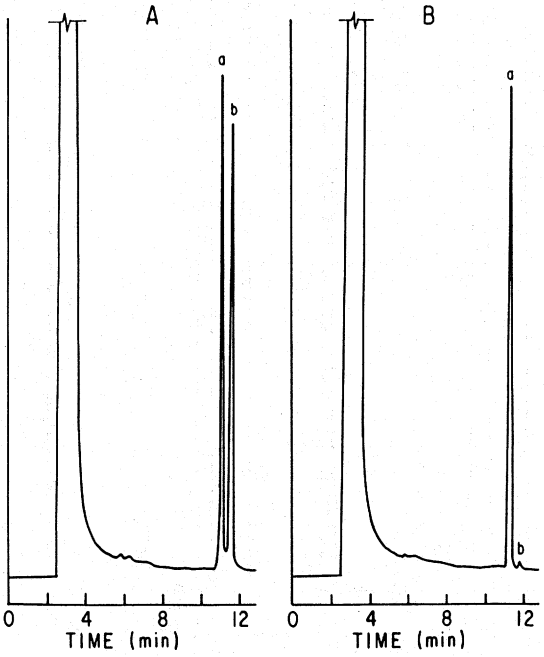

FIG. 2. (A) Analysis of α-methylbenzyl carbamates of (±)-8-methyl-2-decanol formed by reaction with *S*-α-methylbenzyl isocyanate (SPB-1, 240°C). Peak a is the *RS* diastereomer, and b is the *SS* diastereomer. (B) Analysis of 8-methyl-2-decanol obtained by hydrolysis of the racemic octanoate ester using *M. miehei* lipase (Novo-225). Peak a is the *RS* diastereomer, and the major product of lipolytic hydrolysis has the *R* configuration

sources of acid residues were conducted neat (Methods and Materials). The degree of conversion was estimated by gas chromatography and distillation to recover alcohol. Results are presented in Table 7; only the lipases of *C. rugosa* and *M. miehei* gave reasonable conversions. The most striking observation was that the addition of a small amount of water to reaction mixtures containing the Novo *M. miehei* lipase, but not the other lipases, produced a profound increase in stereoselection. It may be that in a nonaqueous medium the enzyme is deprived of water at the catalytic site, water that if present may be held in such manner that the enzyme operates stereoselectively.

Application. The transesterifications using olive oil and tallow had not shown good selectivity, even with Novo's *M. miehei* lipase. Therefore preparative reactions were limited to hydrolysis and esterification. The octanoate ester of 2-octanol was allowed to react in water at pH 7.0 to 11% conversion

using Novo *M. miehei*. The 2-octanol recovered by distillation, 10% yield (91% of theory based on conversion), was 99% *R*. Similarly, the octanoate ester of 8-methyl-2-decanol was allowed to react to 15% conversion and yielded (10%) 98.7% 2-*R* isomer. The rate ratio for this ester is therefore ca. 95. Employing the currently available liquid form of this lipase, Novo-225, a 26% yield of 98.8% 2-*R* isomer was obtained implying a somewhat improved stereobias. These reactions had been allowed to proceed at room temperature for 24–40 hr.

Esterification of racemic 8-methyl-2-decanol in hexane with octanoic acid was conducted with Novo's lipase 3A, which is derived from *M. miehei* and is formulated on an ion exchange resin. Because that rate ratio is again fairly high (ca. 100), and the resin-borne enzyme is very stable, it is possible to produce both *R* and *S* alcohols with high configurational purities and in good yields. Reactions were allowed to proceed to nearly 50% conversion and the recovered ester was saponified to *R* alcohol. Adjustments (reduction) in percent conversion can be made to provide a purer *R* ester; a higher conversion would result in an alcohol richer in the *S* configuration. In a particular experiment, 41–42% yields of each alcohol were obtained: 94.6% *R* and 93.2% *S*; in another, similar yields were obtained: 95.4% *R* and 91% *S*.

These processes represent resolutions of aliphatic alcohols that are among the more difficult structural types to resolve. Simple secondary alcohols frequently find use in organic syntheses and occasionally have important biological activity. Since the *M. miehei* lipase is commercially available, and the procedures employed are quite simple, these results should prove quite useful.

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